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Supporting Information

Multinuclear Non-Heme Iron Complexes for Double Strand DNA Cleavage

Rik P. Megens, Tieme A. van den Berg, A. Dowine de Bruijn, Ben L. Feringa* and Gerard Roelfes*[a]

Stratingh Institute for Chemistry
University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)

Rik P. Megens, Tieme A. van den Berg, A. Dowine de Bruijn, Ben L. Feringa* and Gerard Roelfes*

Stratingh Institute for Chemistry
University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
Fax: (+31) 50-363-4296
E-mail: j.g.roelfes@rug.nl; b.l.feringa@rug.nl

Instrumentation

NMR spectra were recorded on a Varian Mercury Plus 200 (^{1}H NMR at 200 MHz, ^{13}C NMR at 50.3 MHz), a Varian VXR-300 (^{1}H NMR at 300 MHz, ^{13}C NMR at 75.5 MHz), or on a Varian Mercury Plus 400 (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz). Chemical shifts (d) are denoted in ppm and referenced to the residual solvent peak unless stated otherwise ($CDCl_3$, ^{1}H d = 7.24, ^{13}C d = 77.0; CD_3OD, ^{1}H d = 3.30, ^{13}C d = 49.0; CD_3CN, ^{1}H d = 1.93, D_2O ^{1}H d = 4.79). The splitting patterns are designated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants (*J*) between two nuclei separated by n chemical bonds are denoted in hertz (Hz).
Chemical ionisation mass spectra (MS-CI$^+$), electron impact (MS-EI$^+$), and exact mass determination (HRMS) were recorded on a AEI MS-902. Electrospray ionisation mass spectrometry (MS-ESI$^+$) was performed on a Triple Quadrupole LC-MS-MS mass spectrometer (API 3000, Perkin-Elmer Sciex Instruments).
Elemental analysis was performed on an EuroVector CHNS-O Elemental Analyzer Euro EA 3000. Melting points were recorded on a Büchi B-545 melting point apparatus. UV-Vis spectra were recorded on a Hewlett-Packard 8453 diode array or Jasco V-570 spectrophotometer. High resolution ESI-MS was performed on a Applied Biosystems Q-STAR mass spectrometer.

HPLC separations

Reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on a Shimadzu LC-10AD vp at 35°C, which was equipped with a photodiode array detector (SPD M10A) and a fraction collector (Shimadzu FRC 10A). A Waters 2690 Separations Module equipped with a photodiode array detector (Waters 996) and a Micromass ZMD Quadrupole mass spectrometer at room temperature was used for LC-MS analysis.

Analytical HPLC analysis

Method A; A Waters XTerra MS C18 analytical column (3.5 µm, 3.0× 150 mm) was used with a flow rate of 0.5 mL/min. A linear gradient of water (containing 0.05% acetic acid) and methanol (containing 0.05% acetic acid) was used, going from 10% organic solvent to 70% organic solvent over a period of 60 min.
Method B; A Waters Xbridge C18 analytical column (3.5 µm, 4.6× 150 mm) was used with a flow rate of 0.8 mL/min. A linear gradient of 10 mM ammonium acetate (pH =5.5) and methanol was used, going from 40% to 60% organic solvent over a period of 45 min.

Purification of compounds with preparative HPLC

Ligands **2-4**, **7** and **8** were purified on a Waters XTerra MS C18 preparative column (3.5 µm, 4.6 x 150 mm) was used with a flow rate of 0.5 mL/min. A linear gradient of water (containing 0.05% acetic acid) and methanol (containing 0.05% acetic acid) was used, going from 10% organic solvent to 70% organic solvent over a period of 60 min.
Ligands **5** and **6** were purified on a Waters Xbridge C18 preparative column (3.5 µm, 4.6 x 150 mm) was used for this purpose at a flow rate of 10 mL/min. A linear gradient of 10 mM ammonium acetate (pH = 5.5) and methanol was used, going from 40% to 60% organic solvent over a period of 45 min. The fractions were analysed using analytical HPLC with method A or B. After pooling the fractions containing pure material, the resulting solution was concentrated, extracted with CH_2Cl_2 and dried over Na_2SO_4. Filtration and evaporation of the solvent yielded pure material.

1-[(2-{[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}benzoyl)oxy]-2,5-pyrrolidinedione (10)
To a cooled (0°C) solution of *N*-hydroxysuccinimide (1.27 g; 11.0 mmol) in THF (30 mL) was added triethyl amine (1.11 g; 1.52 mL; 11.0 mmol) and freshly distilled *o*-phthaloyl dichloride (1.02 g; 0.73 mL; 5.00 mmol). The resulting white suspension was stirred for 2 hours at room temperature. The solvent was removed and the residue was taken up in dichloromethane (100 mL), washed with water (3× 50 mL) and dried over Na_2SO_4. Filtration and evaporation of the solvent yielded an off-white solid, which was recrystallized from isopropyl alcohol to yield a white solid (1.40 g; 3.90 mmol; 78%).
M.p. 161.9-162.0°C.
^{1}H NMR (300 MHz, $CDCl_3$) 8.01-8.06 (m, 2H), 7.71-7.78 (m, 2H), 2.85 (s, 8H).
^{13}C NMR (50.3 MHz, $CDCl_3$) 168.7, 161.5, 133.2, 130.9, 127.3, 25.7.
MS-CI$^+$: *m/z* 378.1 [M + NH_4^+]$^+$ (100%).
Anal. calcd (%) for $C_{16}H_{12}N_2O_8$: C 53.34, H 3.36, N 7.78; found: C 53.0, H 3.34, N 7.68.

1-[(3-{[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}benzoyl)oxy]-2,5-pyrrolidinedione (11)

Following the procedure as for **10** starting from isophthalic acid (2.00 g; 12.0 mmol), DCC (5.52 g; 26.5 mmol) and *N*-hydroxysuccinimide (3.05 g; 26.5 mmol) in THF (300 mL). A white solid (3.78 g; 10.5 mmol; 86%) was obtained after recrystallization from isopropyl alcohol. M.p. 255°C (decomp.).
^{1}H NMR (400 MHz, $CDCl_3$) 8.90 (t, J = 1.4 Hz, 1H), 8.44 (dd, J = 7.9 Hz, J = 1.4 Hz, 2H), 7.72 (t, J = 7.9 Hz, 1H), 2.93 (s, 8H).
^{13}C NMR (50.3 MHz, $CDCl_3$) 168.8, 160.6, 136.3, 132.4, 129.7, 126.3, 25.6.
MS-CI$^+$: *m/z* 378.1 $[M + NH4^+]^+$.
Anal. calcd (%) for $C_{16}H_{12}N_2O_8$: C 53.34, H 3.36, N 7.78; found: C 53.5, H 3.35, N 7.75.

1-[(4-{[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}benzoyl)oxy]-2,5-pyrrolidinedione (12)
Following the procedure as for **10**, starting from terephthalic acid (2.01 g; 12.1 mmol), DCC (5.49 g; 26.6 mmol) and *N*-hydroxysuccinimide (3.06 g; 26.6 mmol) in THF (300 mL). A white solid (3.74 g; 10.4 mmol; 86%) was obtained after recrystallization from isopropyl alcohol. M.p. 260°C (decomp.).
^{1}H NMR (400 MHz, $CDCl_3$) 8.26 (s, 4H), 2.92 (s, 8H).
^{13}C NMR (100 MHz, $CDCl_3$) 168.75, 160.83, 130.81, 130.66, 25.68.
MS-CI$^+$: *m/z* 378.1 $[M + NH4^+]^+$ (100%).
Anal. calcd (%) for $C_{16}H_{12}N_2O_8$: C 53.34, H 3.36, N 7.78; found: C 53.4, H 3.44, N 7.73.

1-[(3,5-bis{[(2,5-dioxo-1-pyrrolidinyl)oxy]carbonyl}benzoyl]-2,5-pyrrolidinedione (13)
A mixture of 1,3,5-benzene tricarboxylic acid (1.01 g; 4.79 mmol), *N*-hydroxysuccinimide (1 82 g; 15.8 mmol) and DCC (3.30 g; 16.01 mmol) in THF (250 ml) was stirred overnight. The white suspension was filtered and the supernatant was concentrated. The resulting white solid was recrystallized from isopropanol. The product was obtained as a white solid (1.82 g; 3.64 mmol; 76%). M.p.. 255°C (decomp.).
^{1}H NMR (400 MHz, $CDCl_3$) 9.14 (s, 3H), 2.94 (s, 12H).
^{13}C NMR (100 MHz, $CDCl_3$) 168.50, 159.54, 137.36, 127.67, 25.61.
MS-CI$^+$: *m/z* 388 $[M – NHS + H^+]^+$.
Anal. calcd (%) for $C_{21}H_{15}N_3O_{12}$: C 50.3; H 3.02, N 8.38; found: C 50.1; H 3.12; N 8.28.

6-benzyl 1-ethyl 3-[3[(benzyloxy)-3-oxopropyl]-2-hexenedioate (15)
Triethyl phosphonoacetate (3.1 g; 13.84 mmol) in THF (10 mL) was added slowly to a cooled solution of NaH (332 mg; 13.84 mmol) in THF (20 mL). The resulting reaction mixture was stirred for an additional 30 min at 0°C, before benzyl protected 4-ketopimelic acid (**14)** (4.46 g; 12.58 mmol) was added. The resulting mixture was stirred for 1 h at room temperature and heated under reflux overnight. Water (20 mL) was added and the mixture was extracted with EtOAc (3× 20 mL). The organic layers were dried (Na_2SO_4), filtered and the solvent was evaporated. The product was further purified by column chromatography (SiO_2; EtOAc: Pentane; 1: 10) to afford the product **15** (2.89 g; 6.82 mmol; 54%) as a white solid.
^{1}H NMR (400 MHz, $CHCl_3$) 7.36 (m, 10H), 5.63 (s, 1H), 5.09 (s, 4H), 4.11 (q, J = 7.0 Hz, 2H), 2.87 (t, J = 6.2 Hz, 4H), 2.50 (t, J = 6.2 Hz, 4H), 1.24 (t, J = 7.0 Hz, 3H).
^{13}C NMR (50.4 MHz, $CDCl_3$): d 206.8, 206.6, 172.5, 172.3, 135.7, 128.4, 128.1, 128.0, 66.3, 60.5, 36.9, 36.9, 31.8, 29.6, 29.2, 27.8, 27.6, 22.5, 14.0.
MS-CI$^+$: *m/z* 442 $[M + NH_4^+]^+$ (100%), 425 $[M + H^+]^+$ (100%).

4-(2-ethoxy-2-oxoethyl)heptanedioic acid (16)
To a solution of **15** (1.00 g, 2.36 mmol) in MeOH (25 mL) was added Pd/C (10%, 0.1 g) and the suspension was stirred under a H_2 atmosphere overnight. The mixture was filtered over Celite®. After washing the filtercake with MeOH, the solvent was evaporated to afford the reduced diacid **16** (0.54 g; 2.34 mmol; 99%) as a solid.
^{1}H NMR (400 MHz, $CHCl_3$) 4.12 (q, J = 7.1 Hz, 2H), 2.23-2.47 (m, 6H), 1.90 (m, 1H), 1.66 (m, 4H), 1.23 (t, J = 7.0 Hz, 3H)
^{13}C NMR (50.3 MHz, $CDCl_3$) 177.15, 170.54, 59.51, 38.35, 33.98, 31.21, 28.56,
HRMS (EI$^+$) calcd. for $C_{11}H_{17}O_5$ $(M - OH^-)^+$: *m/z* 229.107; found: 229.106.

4-(carboxymethyl)heptanedioic acid (17)
A mixture of **16** (0.21 g; 0.85 mmol) and LiOH (61.33 mg; 2.56 mmol) in MeOH/H_2O (5 mL; 1:1 v/v) was stirred overnight. The mixture was washed with CH_2Cl_2 (10 mL) and conc. H_2SO_4 was added until pH ~ 2. The solution was extracted with EtOAc (12× 10 mL). After drying (Na_2SO_4) and filtration the product was isolated as a white solid (105 mg; 0.48 mmol; 56%).
1H NMR (300 MHz, CD_3OD)?2.33 (t, J = 7.80, Hz, 4H), 2.27 (d, J = 6.63 Hz, 5H), 1.89 (td, J = 12.94, 6.47 Hz, 1H), 1.65 (m, 4H)
^{13}C NMR (100 MHz, $CDCl_3$) 205.4, 204.6, 67.1, 63.3, 60.1, 57.7.
HRMS (EI$^+$)calcd. for $C_9H_{13}O_5$ $(M - OH^-)^+$: *m/z* 201.076; found: 201.076.

Dimethyl 5-{3-[(*tert*-butoxycarbonyl)amino]propoxy}isophthalate (**21**)
Dimethyl 5-hydroxyisophthalate (1.00 g; 4.73 mmol) was dissolved in acetone (50 mL). To this solution the Boc-protected bromopropylamine **20** (0.563 g; 2.36 mmol) and K_2CO_3 (0.980 g; 7.09 mmol) were added and the white suspension was heated under reflux overnight. After cooling to room temperature the suspension was filtered and the solvent was removed. CH_2Cl_2

(50 mL) was added and after an additional filtration, the solution was washed with sat. aq. $NaHCO_3$ (2× 50 mL) and water (50 mL). Drying (Na_2SO_4), filtration and evaporation yielded a colorless oil. After purification by column chromatography (SiO_2; pentane: EtOAc 4:1 to 1:1; R_f = 0.25) a white solid was obtained (0.505 g; 1.37 mmol; 58%). M.p. 76.8-77.0°C.
^{1}H NMR (200 MHz, $CDCl_3$) 8.26 (t, J = 1.4 Hz, 1H), 7.72 (d, J = 1.4 Hz, 2H), 4.88-4.58 (br. s, 1H), 4.09 (t, J = 6.0 Hz, 2H), 3.91 (s, 6H), 3.33 (m, 2H), 2.01 (m, 2H), 1.43 (s, 9H).
^{13}C NMR (50.3 MHz, $CDCl_3$) 166.0, 158.8, 155.9, 131.7, 123.0, 119.7, 79.3, 66.2, 52.3, 37.8, 29.4, 28.3.
HRMS (EI^+) calcd. for $C_{18}H_{25}NO_7$: *m/z* 367.163 ; found 367.164
Anal. calcd (%) for $C_{18}H_{25}NO_7$: C 58.84, H 6.86, N 3.81; found: C 59.0, H 6.78, N 3.61.

5-{3-[(*tert*-butoxycarbonyl)amino]propoxy}isophthalic acid (**18**)
To a solution of the diester **21** (0.525 g; 1.43 mmol) in THF/H_2O (20 mL; 1:1 v/v) was added LiOH (0.103 g; 4.29 mmol). The mixture was stirred at room temperature for 48 h. Conc. H_2SO_4 (96%; 0.24 mL; 4.3 mmol) was added, resulting in a white suspension, which became clear after standing overnight (pH < 1). The solution was extracted with CH_2Cl_2 (4× 10 mL). The combined organic fractions were washed with water (3× 10 mL) and dried over Na_2SO_4. Filtration and evaporation yielded a white solid (0.404 g; 1.19 mmol; 83%). M.p. 270.6-272.4°C (decomp.).
^{1}H NMR (400 MHz, DMSO-d_6) 8.04 (t, J = 1.4 Hz, 1H), 7.60 (d, J = 1.4 Hz, 2H), 6.88 (t, J = 5.2 Hz, 1H), 4.05 (t, J = 6.2 Hz, 2H), 3.07 (m, 2H), 1.81 (m, 2H), 1.34 (s, 9H).
^{13}C NMR (50.3 MHz, DMSO-d_6) 166.4, 158.8, 155.6, 132.6, 122.1, 119.0, 77.5, 65.9, 36.7, 29.1, 28.2.
HRMS (EI^+) calcd. for $C_{16}H_{21}NO_7$: *m/z* 339.132; found: 339.132
Anal. calcd (%) for $C_{16}H_{21}NO_7$: C 56.6, H 6.24, N 4.13; found: C 56.7, H 6.25, N 4.15.

Dimethyl-2-methoxyisophtalate (22)
To a solution of 2-methoxyisophtalic acid (5.07 g; 25.8 mmol) in 250 mL MeOH was added 1 mL H_2SO_4. The solution was heated under reflux overnight. After cooling to room temperature the solvents were removed and 50 mL CH_2Cl_2 was added. The solution was washed with H_2O (2× 50 mL) and dried over Na_2SO_4. Evaporation of the solvent and purification by column chromatography (SiO_2, pentane: EtOAc 9:1 → 5:1 → 3:1) gave **1** (4.37 g, 19.5 mmol, 75%) as a yellow oil.
^{1}H-NMR (400 MHz, $CDCl_3$) 7.94 (d, J = 7.7, 2H), 7.21 (t, J = 7.7, 1H), 3.93 (s, 9H).
^{13}C NMR (50.3 MHz, $CDCl_3$) 166.0, 159.5, 134.9, 126.5, 123.4, 63.6, 52.3.
HRMS calcd. for $C_{11}H_{12}O_5$: *m/z* 224.0684, found: 224.0671.

Dimethyl-2-hydroxyisophtalate (23)
A solution of **22** (2.60 g; 11.6 mmol) in 50 mL dry CH_2Cl_2 was placed under N_2-atmosphere and cooled to -78°C. Slowly 1 M BBr_3 in CH_2Cl_2 (12.7 mL; 12.7 mmol) was added. After stirring for 45 minutes at -78°C, 15 mL MeOH was added and the solution was warmed up to room temperature. The mixture was washed with sat. aq. $NaHCO_3$(3× 30 ml) and dried over Na_2SO_4. Evaporation of the solvent gave **23** (1.57 g; 64%) as a yellow oil.
^{1}H-NMR ($CDCl_3$) 11.81 (s, 1H), 8.03 (d, J = 7.9, 2H), 6.92 (t, J = 7.9, 1H), 3.93 (s, 6H).
^{13}C-NMR (50.3 MHz, $CDCl_3$) 168.0, 161.5, 136.3, 118.4, 116.4, 52.4.
HRMS calcd. for $C_{10}H_{10}O_5$: 210.0528; found: 210.0523.
Anal. calcd. $C_{10}H_{10}O_5$: C, 57.14, H, 4.80: found C, 56.91, H, 4.79.

Dimethyl-2-(3-(tert-butoxycarbonylamino)propoxy)isophtalate (24)
To a solution of **23** (1.1 g; 4.7 mmol) and K_2CO_3 (3.24 g; 23.5 mmol) in 150 mL DMF was added a solution of **20** (1.0 g; 4.7 mmol) in 10 mL DMF. After heating overnight, the solution was decanted. The solvent was removed and 50 mL CH_2Cl_2 was added. The mixture was washed with brine (3× 20 mL) and H_2O (3× 20 mL) and dried over Na_2SO_4. Evaporation of the solvent and purification by column chromatography (SiO_2; pentane: EtOAc 5:1 → 3:1) gave **24** as a yellow oil (1.30 g; 3.5 mmol; 75%).
^{1}H-NMR (400 MHz, $CDCl_3$) 7.95 (d, J = 7.8, 2H), 7.18 (d, J = 7.8, 1H), 5.49 (s, 1H), 4.07 (t, J = 5.6, 2H), 3.92 (s, 6H), 3.38 (q, J = 5.9, 11.9 Hz, 2H), 1.96 (m, 2H), 1.45 (s, 9H).
^{13}C-NMR (50.3 MH, $CDCl_3$) 166.1, 158.8, 156.4, 135.4, 126.8, 123.8, 78.9, 74.2, 52.8, 37.6, 30.1, 28.7.
MS (CI^+, NH_3) *m/z* 385 ($[M + NH_3]^+$, 100), 368 (71), 329 (49), 268 (19).

2-(3-(tert-butoxycarbonylamino)propoxy)isophtalic acid (19)
To a solution of **23** (0.92 g; 2.37 mmol) in 20 mL THF and 20 mL H_2O was added LiOH (212 mg; 710 mmol). After stirring at room temperature for 24 hours, conc. H_2SO_4 (3.5 mL; 710 mmol) was added. The solution was extracted with CH_2Cl_2 (5× 20 mL), washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave **19** as a white solid (300 mg; 0.88 mmol, 68%). M.p. 148-149°C.
^{1}H-NMR (400 MHz, DMSO) 7.80 (m, 2H) , 7.25 (m, 1H), 6.75 (s, 1H) , 3.95 (t, J = 6.0 Hz, 2H), 3.09 (q, J = 6.6, 13.4 Hz, 2H) , 1.78 (m, 2H), 1.37 (s, 9H).
^{13}C-NMR (50.3 MHz, DMSO) 167.8, 157.0, 156.2, 134.1, 128.6, 124.3, 78.2, 74.3, 38.9, 31.0, 28.9.
MS-CI^+: *m/z* 357 $[M + NH_4^+]^+$, 340 $[M + H^+]^+$.
Anal. calcd. (%) for $C_{16}H_{21}NO_7$: C 56.6, H 6.24, N 4.13; found: C 57.1, H 6.47, N 4.02.

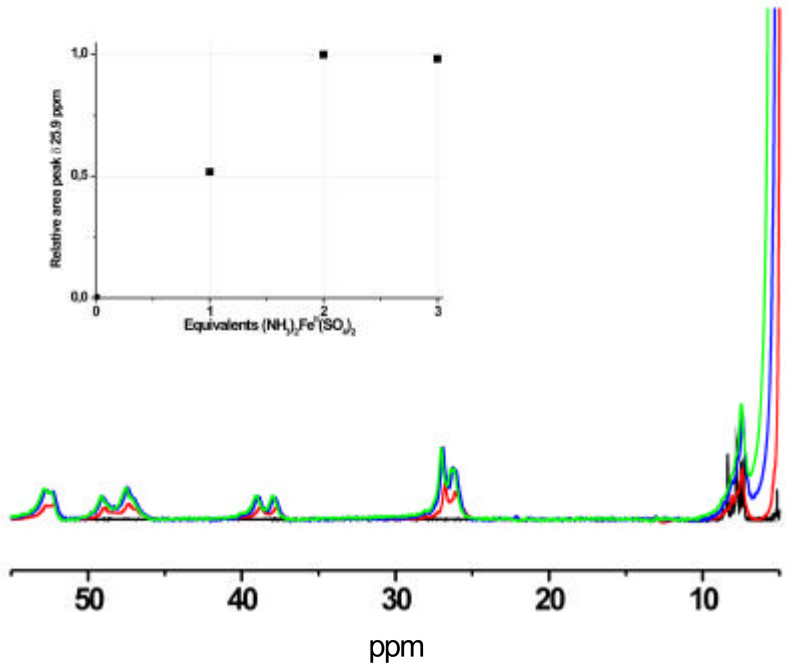


Figure S1. ^{1}H-NMR titration of **3** in D_2O with $(NH_3)_2Fe^{II}(SO_4)_2$ (—) 0 eq. (—) 1 eq. (—)2 eq. (—) 3 eq. of $(NH_3)_2Fe^{II}(SO_4)_2$. Inset; plot of the relative area of the peak at d 25.9 ppm compared to the acetone peak against the amount of equivalents of $(NH_4)_2Fe^{II}(SO_4)_2$.

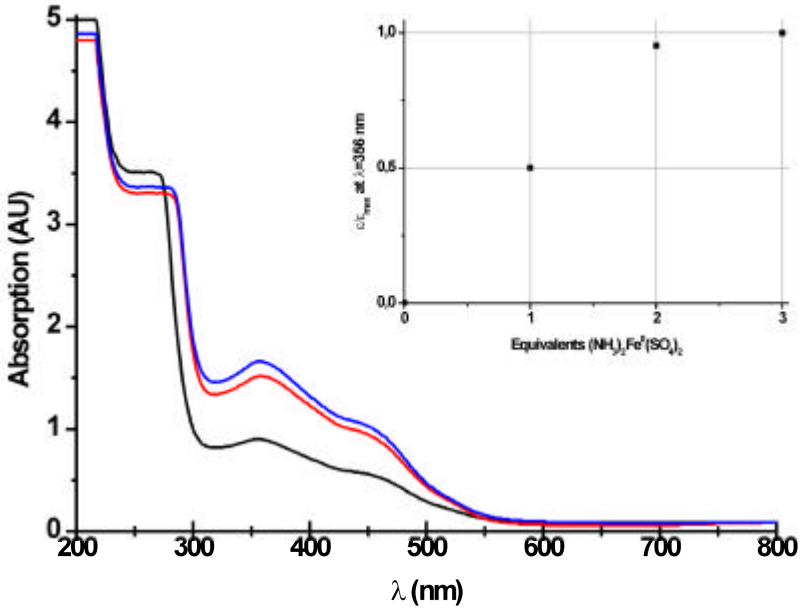


Figure S2. UV/Vis spectra of ligand **3** after addition of (—) 1 eq. (—) 2 eq. (—) 3 eq. of $(NH_3)_2Fe^{II}(SO_4)_2$. Inset; plot of the e/e_{max} at 356 nm against the amount of equivalents of $(NH_4)_2Fe^{II}(SO_4)_2$.

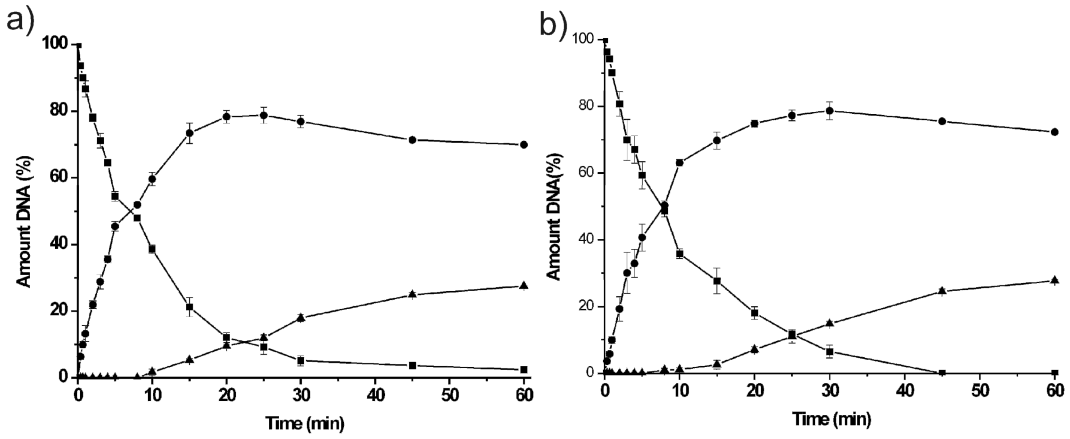


Figure S3 Aerobic oxidation of supercoiled plasmid DNA (¦) into nicked DNA (?) and linear DNA (?) followed in time catalyzed by a) [[(**2**)Fe^{II}_2]$^{4+}$ (**30**) and b) [(**4**)Fe^{II}_2]$^{4+}$ (**32**). Errors bars represent the root mean square (rms) error based on three runs. A correction factor of 1.31 was used to compensate for the reduced ethidium bromide uptake capacity of supercoiled DNA

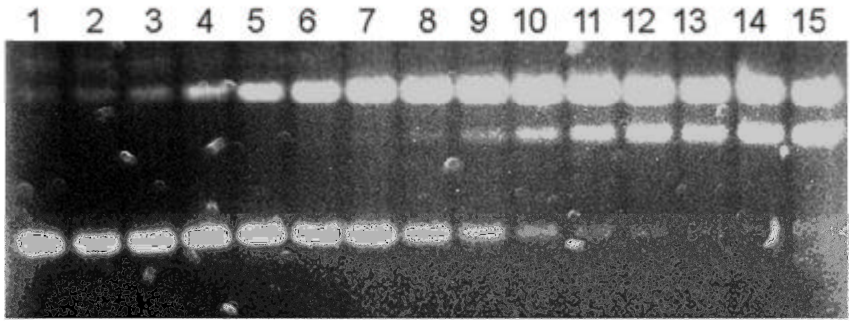
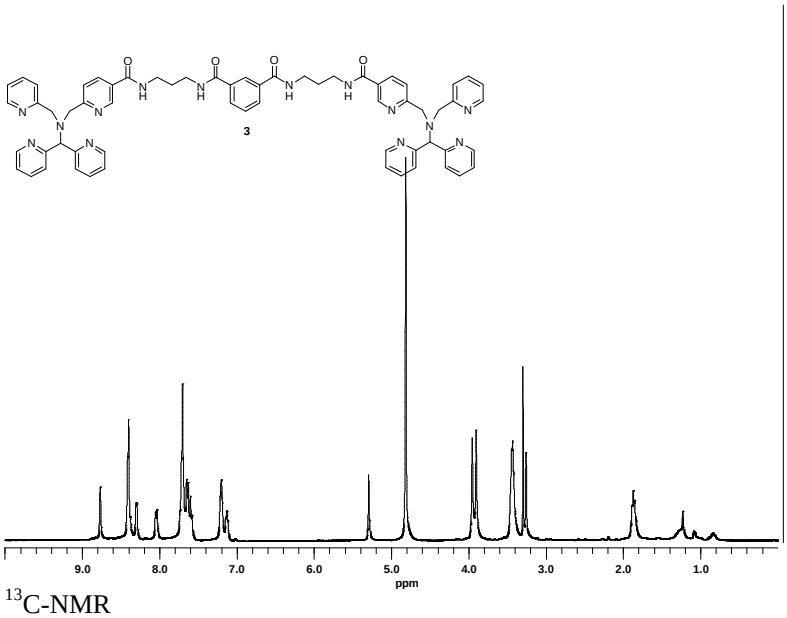
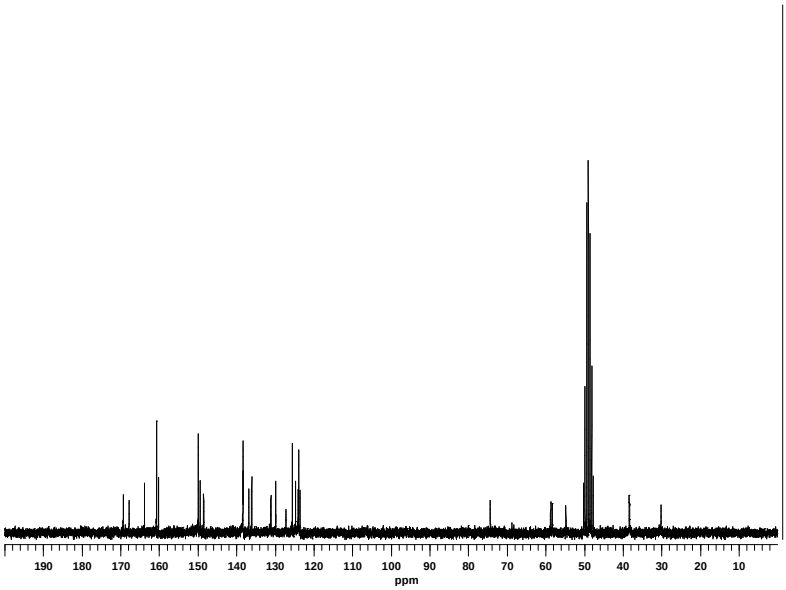


Figure S4: Example of an agarose gel slab of the reaction of supercoiled DNA to form nicked and linear DNA with complex **32**. Each lane represents a surtain time point in the time profile plots; lane 1, 20s; lane 2, 40s; lane 3, 1 min.; lane 4, 2 min.; lane 5, 3 min.; lane 6, 4 min.; lane 7, 5 min.; lane 8, 7.5 min.; lane 9, 10 min.; lane 10, 15 min; lane 11, 20 min.; lane 12, 25 min.; lane 13, 30 min.; lane 14, 45 min; lane 15, 60 min.

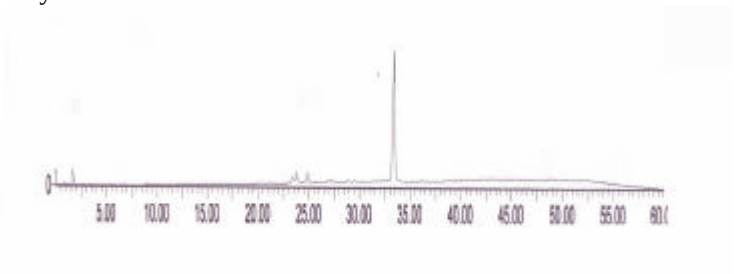
^{1}H-NMR



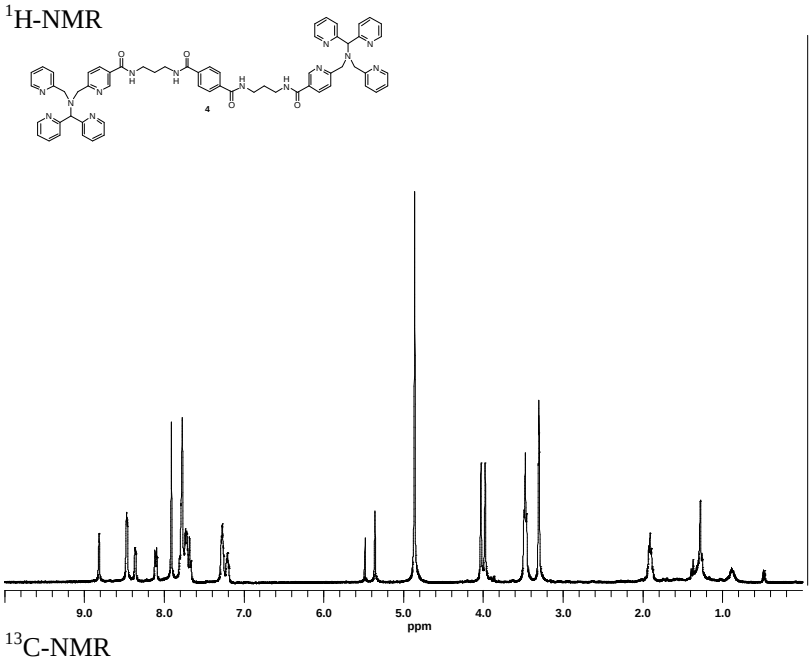
^{13}C-NMR



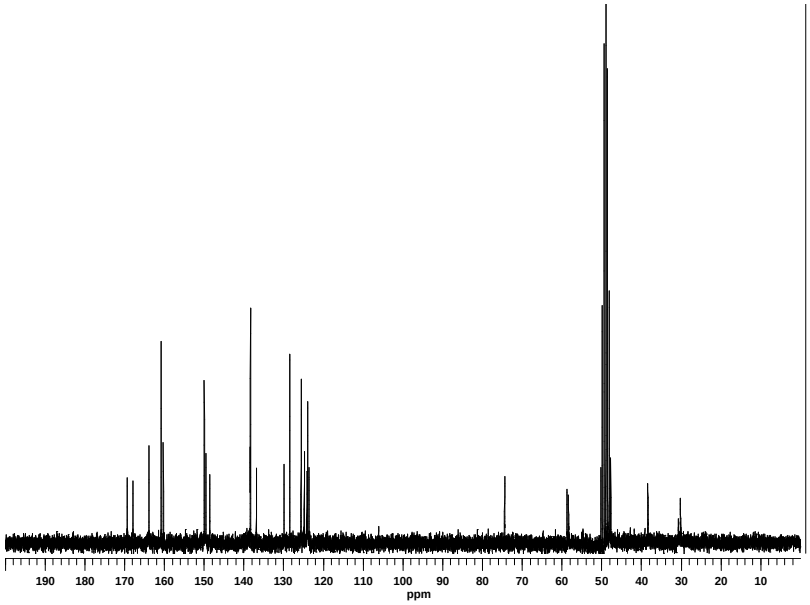
Analytical RP-HPLC



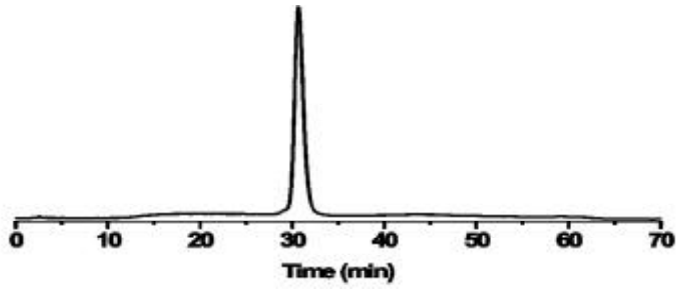
^{1}H-NMR



^{13}C-NMR



Analytical RP-HPLC



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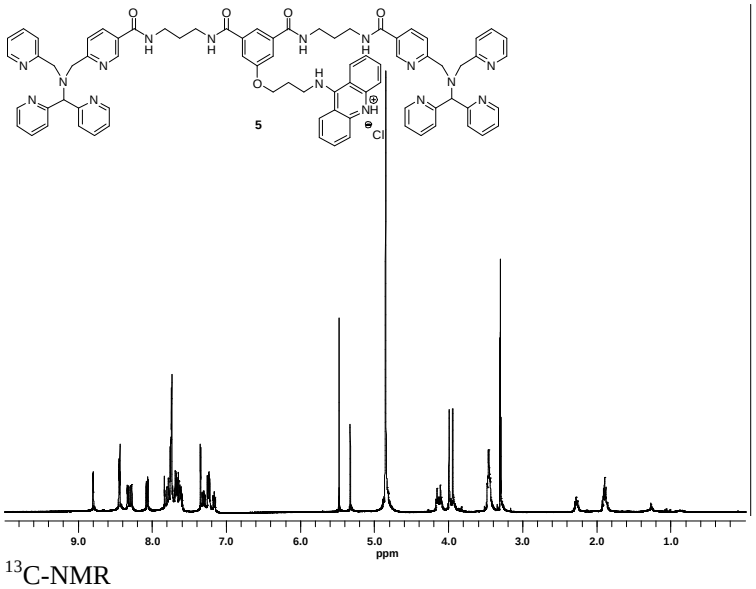
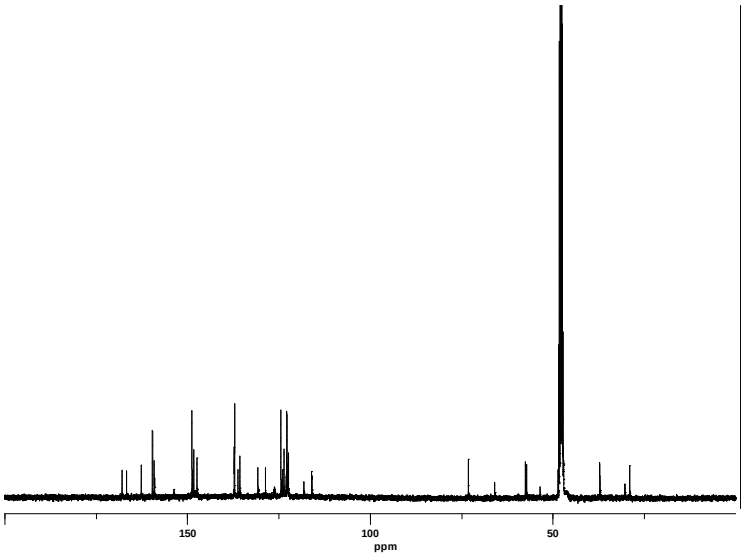


^{13}C-NMR



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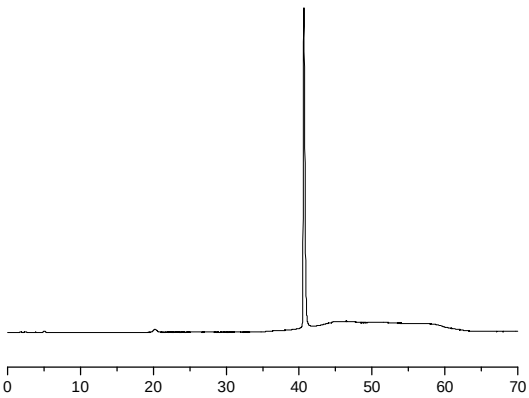

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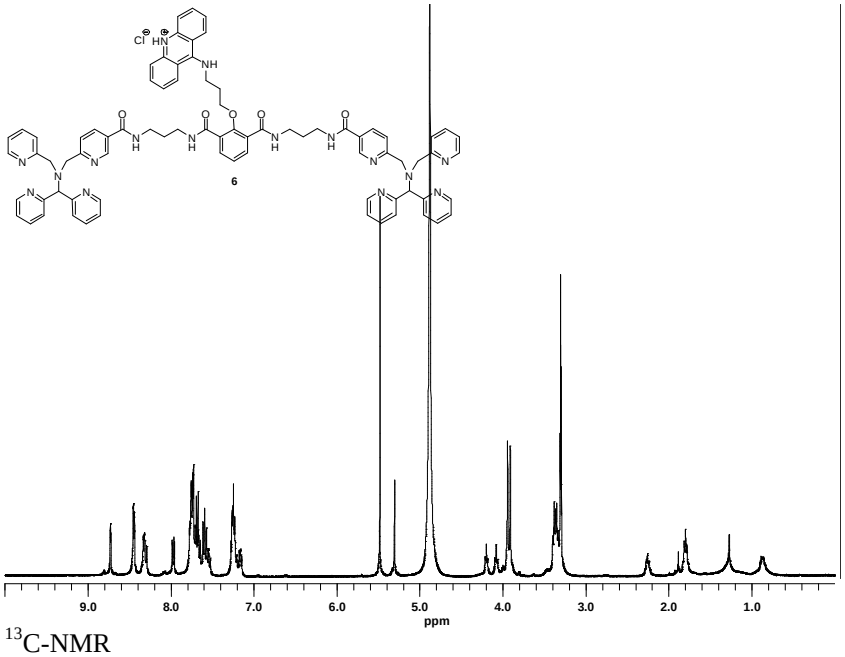

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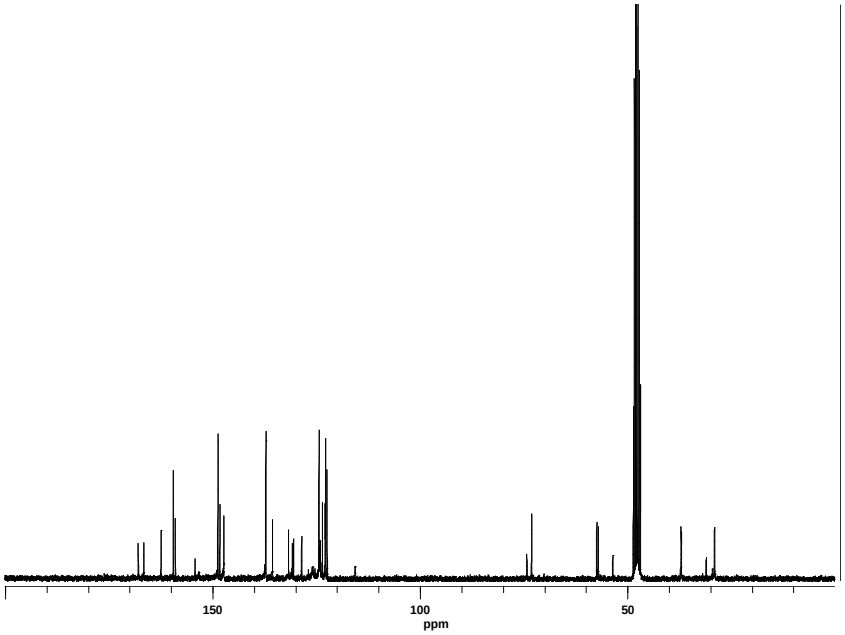

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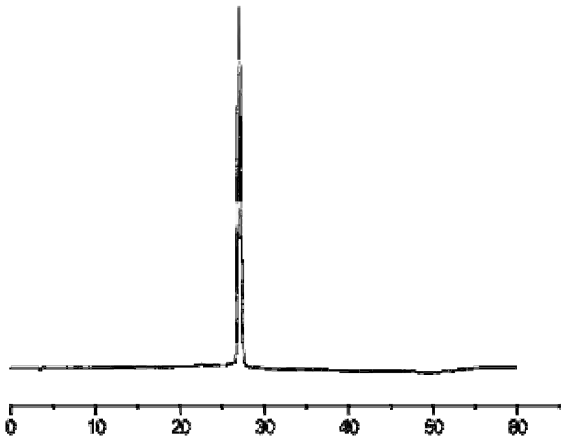

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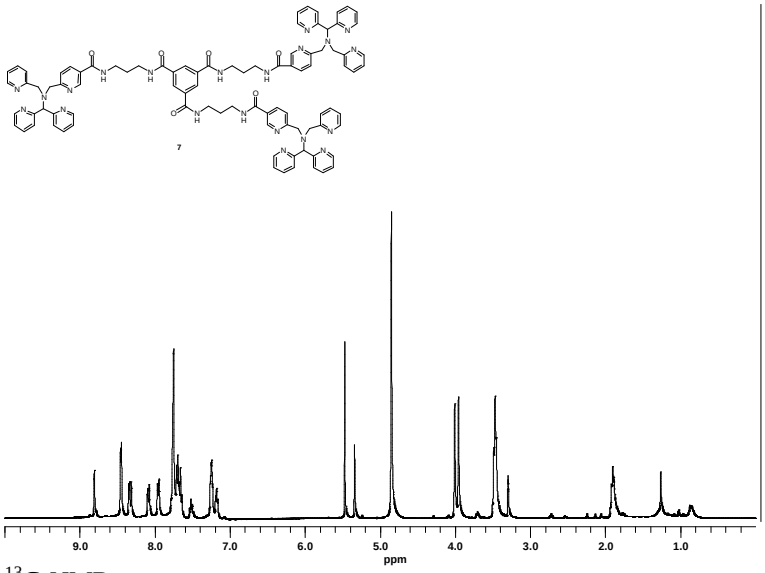

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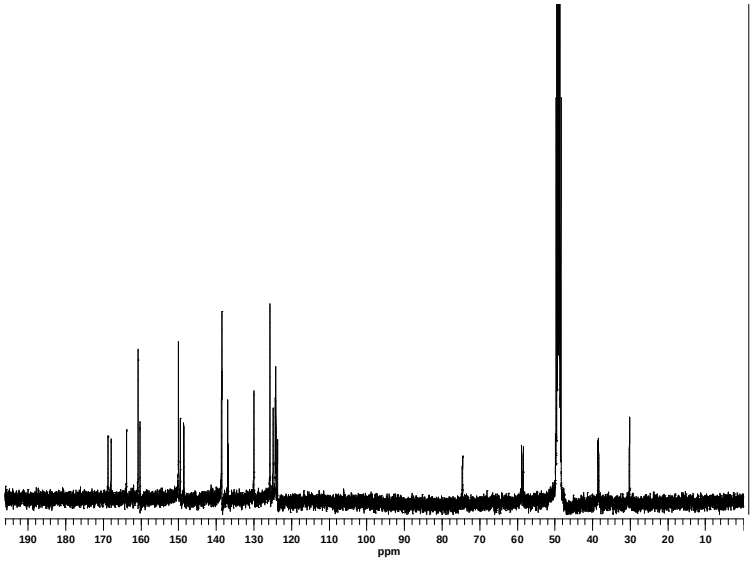

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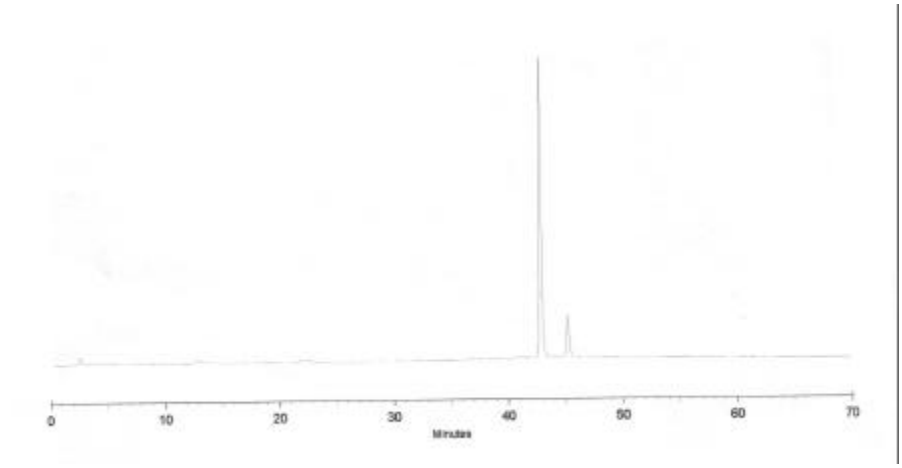

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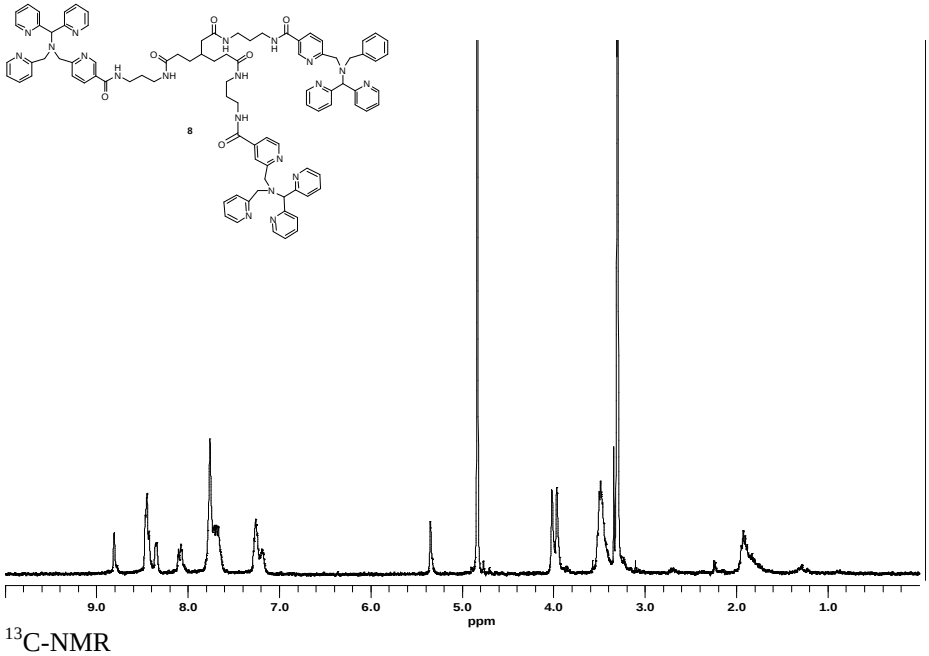

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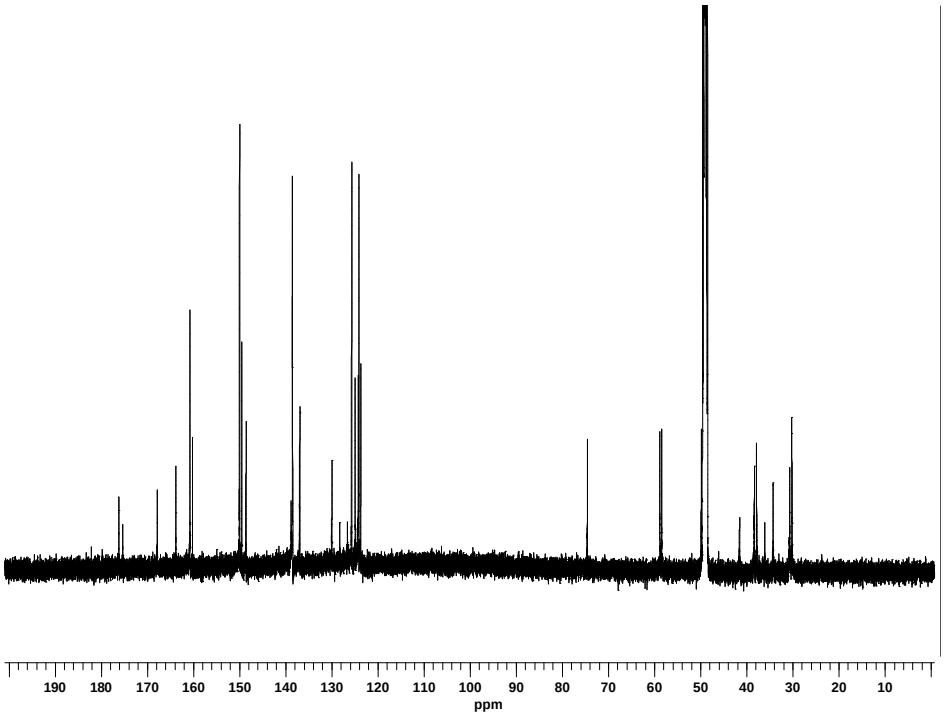

Analytical RP-HPLC